Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus

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Summary

Background **The frequency of asymptomatic infection with Ebola virus is unclear: previous estimates vary and there is no standard test. Asymptomatic infection with Ebola virus could contribute to population immunity, reducing spread. If people with asymptomatic infection are infectious it could explain re-emergences of Ebola virus disease (EVD) without known contact.**

Methods **We validated a new oral fluid anti-glycoprotein IgG capture assay among survivors from Kerry Town Ebola Treatment Centre and controls from communities unaffected by EVD in Sierra Leone. We then assessed the seroprevalence of antibodies to Ebola virus in a cross-sectional study of household contacts of the survivors. All household members were interviewed. Two reactive tests were required for a positive result, with a third test to resolve any discrepancies.**

Findings **The assay had a specificity of 100% (95% CI 98·9–100; 339 of 339 controls tested negative) and sensitivity of 95·9% (89·8–98·9; 93 of 97 PCR-confirmed survivors tested positive). Of household contacts not diagnosed with EVD, 47·6% (229 of 481) had high level exposure (direct contact with a corpse, body fluids, or a case with diarrhoea, vomiting, or bleeding). Among the contacts, 12·0% (95% CI 6·1–20·4; 11 of 92) with symptoms at the time other household members had EVD, and 2·6% (1·2–4·7; 10 of 388) with no symptoms tested positive. Among asymptomatic contacts, seropositivity was weakly correlated with exposure level.**

Interpretation **This new highly specific and sensitive assay showed asymptomatic infection with Ebola virus was uncommon despite high exposure. The low prevalence suggests asymptomatic infection contributes little to herd immunity in Ebola, and even if infectious, would account for few transmissions.**

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*Dr Dhan Samuel died in April, 2016.

Introduction

It is not known how frequently asymptomatic Ebola virus infection occurs, yet it could affect the course of epidemics. High rates of asymptomatic infection would reduce incidence through herd immunity, radically altering model predictions of epidemic spread.[1] If those with asymptomatic infection are infectious, perhaps with persistent viral shedding, it would help explain some failures in control and the emergence of new chains of transmission.[2]

The extent of asymptomatic infection is unclear because previous findings have varied widely (eg, from 1% to 46% of household contacts),[3,4] with positive results reported in some populations unlikely to have been exposed to filoviruses.[5–7] This finding has led to questions about assay specificity and cross-reactivity for ELISAs as well as for the older immunofluorescence antibody techniques. There is no assay approved by the US Food and Drug Administration, and the need for caution in interpreting Ebola virus antibody serosurveys continues to be emphasised.[8]

A reliable serological test could also help identify missed cases with minor symptoms. Asymptomatic infections and missed symptomatic cases might explain the apparent lower incidence of Ebola virus disease (EVD) in children.[9,10] Diagnosis might be missed in young children,[11] and older children could be less susceptible to developing EVD if infected.[12]

A test for Ebola virus antibodies with high sensitivity and specificity is needed. Taking blood is difficult in an Ebola epidemic, due to both the infection risk and population suspicion. We describe the field validation of a new capture ELISA that detects IgG to Ebola virus glycoprotein in oral fluid,[13] and the results of a large seroprevalence study in Ebola-affected households.

Methods

Participants and data collection

All survivors from Kerry Town Ebola Treatment Centre, Sierra Leone, who were discharged between Nov 22, 2014, and March 27, 2015, and their household members

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Research in context

Evidence before this study

We did a systematic review of studies of seroprevalence of antibodies to Ebola virus. We searched PubMed and Web of Science using the search string "ebola AND (asymptom* OR antibod* OR IgG OR immun* OR ELISA OR serol*) NOT vacc* NOT immuniz* AND (Humans[Mesh])", as well as reference lists (including those of previous reviews) and conference reports from the west Africa epidemic. We last updated the search on July 31, 2016, and used no language restrictions.

Different assays have been used and the specificity of the tests is frequently questioned. Of 50 studies, only six reported results for asymptomatic household contacts, with varying prevalence estimates: 2·5% in the first known Ebola virus outbreak using an immunofluorescence assay; and 1·0% in Uganda, 4·0% in the Democratic Republic of Congo, 6·5% in Sierra Leone, and 21·4% and 45·9% in Gabon, using different ELISAs.

Added value of this study

We present the first field validation of a new assay. It had very high specificity and sensitivity and has the added advantage of being non-invasive so was well accepted. Using this assay we showed that the prevalence of seropositivity to Ebola virus in asymptomatic household contacts, many of whom were highly exposed, was only 2·6%. Additionally, 12% of contacts with some symptoms but never diagnosed with Ebola virus disease were seropositive. In these Ebola-affected households, asymptomatic infections accounted for 2·3% and missed symptomatic infections for 2·6% of all Ebola virus infections.

Implications of all the available evidence

Asymptomatic infection with Ebola virus occurs but given the low seroprevalence seen even in highly exposed individuals, it would not be a major contributor to herd immunity. The availability of a reliable non-invasive assay that is easy to administer and highly acceptable in the field will greatly aid future investigations and interventions, including testing and targeting of vaccines.

(people eating from the same pot), were sought for this study. Interviews were done between July 3, 2015, and Sept 10, 2015, encouraging household members to tell their story as a group, as described elsewhere.[12] For each person in the household who was ill or died of EVD we asked who had helped them and had contact with them. We also asked about exposures outside the household. With additional probing questions, we established the maximum exposure level for each person, including those who had not been ill and those who had died, using predefined levels.[12] The highest level was touching the body of someone who died of EVD, then direct contact with body fluids of a wet case (ie, an EVD case with diarrhoea, vomiting, or bleeding); direct contact with a wet case (including nursing and personal care, sharing a bed); direct contact with a dry case (ie, an EVD case without wet symptoms); indirect contact with a wet case (eg, washing clothes or bed linen); indirect contact with a dry case; minimal contact (eg, shared meals); and no known contact.

Individuals who did not report EVD were asked about symptoms at the time that others in the household had EVD. Those reporting symptoms were classified using the Sierra Leone case definition for probable EVD[14] (ie, either contact plus fever or miscarriage or unexplained bleeding, or contact plus three or more symptoms [of fatigue, headache, loss of appetite, nausea or vomiting, abdominal pain, diarrhoea, muscle or joint pain, sore throat or pain on swallowing, and hiccups]).

Swabs (Oracol, Malvern Medical Developments, Worcester, UK) for oral fluid collection were demonstrated by the field staff and then self-administered, with adults helping children. Each swab was rubbed firmly on the gums for 90 s, sealed, put in a cool box, and transferred daily to a –20°C freezer for storage before processing.

Additionally, we recruited community controls in three neighbourhoods of rural Western Area, Sierra Leone, without known EVD cases (Kent, Tokeh, and York). Community leaders with megaphones asked for volunteers of all ages, and we then excluded any with exposure to Ebola and collected oral swabs as described above.

Individual written informed consent was obtained from all participants (or their parents or guardians for those younger than 18 years) before interview and sample collection. Permission for the study was granted by the Sierra Leone Ethics and Scientific Review Committee and the Ethics Committee of the London School of Hygiene & Tropical Medicine.

Procedures

Oral fluid samples were tested for Ebola virus glycoprotein IgG using a new IgG capture assay based on the EBOV Mayinga GP antigen (rGPδTM [catalogue 0501-016]; IBT Bioservices, Rockville, MD, USA) as described elsewhere.[13] Two positive controls (plasma from a UK EVD survivor infected in Sierra Leone) and four negative controls (plasma from UK donors) were included in each plate. The cutoff for a reactive result was defined per plate as the mean optical density (OD) of the negative controls plus a fixed OD measure (0·1). Since the mean negative OD varied between 0·049 and 0·067 per plate, this is equivalent to 2·5–3 times the mean negative OD. We present normalised ODs (ie, the ratio of the test OD to the cutoff), so results greater than 1 were reactive. All reactive samples from household members and controls, all

unreactive samples from survivors, and a selection of other samples including those closer to the cutoff, were repeated. Samples with discrepant results were retested.

Using this assay, results from paired oral fluid and plasma samples have previously been shown to correlate well in 76 participants in an early phase Ebola vaccine trial in the UK (r=0·68, p<0·0001, two-tailed non-parametric Spearman's correlation);[13] ten EVD survivors tested in Connaught Blood Bank, Sierra Leone (r^2=0·83, linear regression); and 80 EVD survivors from Sierra Leone tested in the UK (r^2=0·78, linear regression) (Tedder RS, unpublished). Using the same cutoff as in our study, 78 of 80 samples from the EVD survivors were positive on serum, of which 76 were positive on oral fluid, giving a sensitivity compared with serum of 97·4% (76 of 78 samples). The two samples negative on both oral fluid and plasma were also negative on competitive and double-antigen bridging ELISAs. Additionally, 44 paired oral fluid and plasma samples from individuals not exposed to Ebola from The Gambia were negative on the capture ELISA using the same protocol (Tedder RS, unpublished).

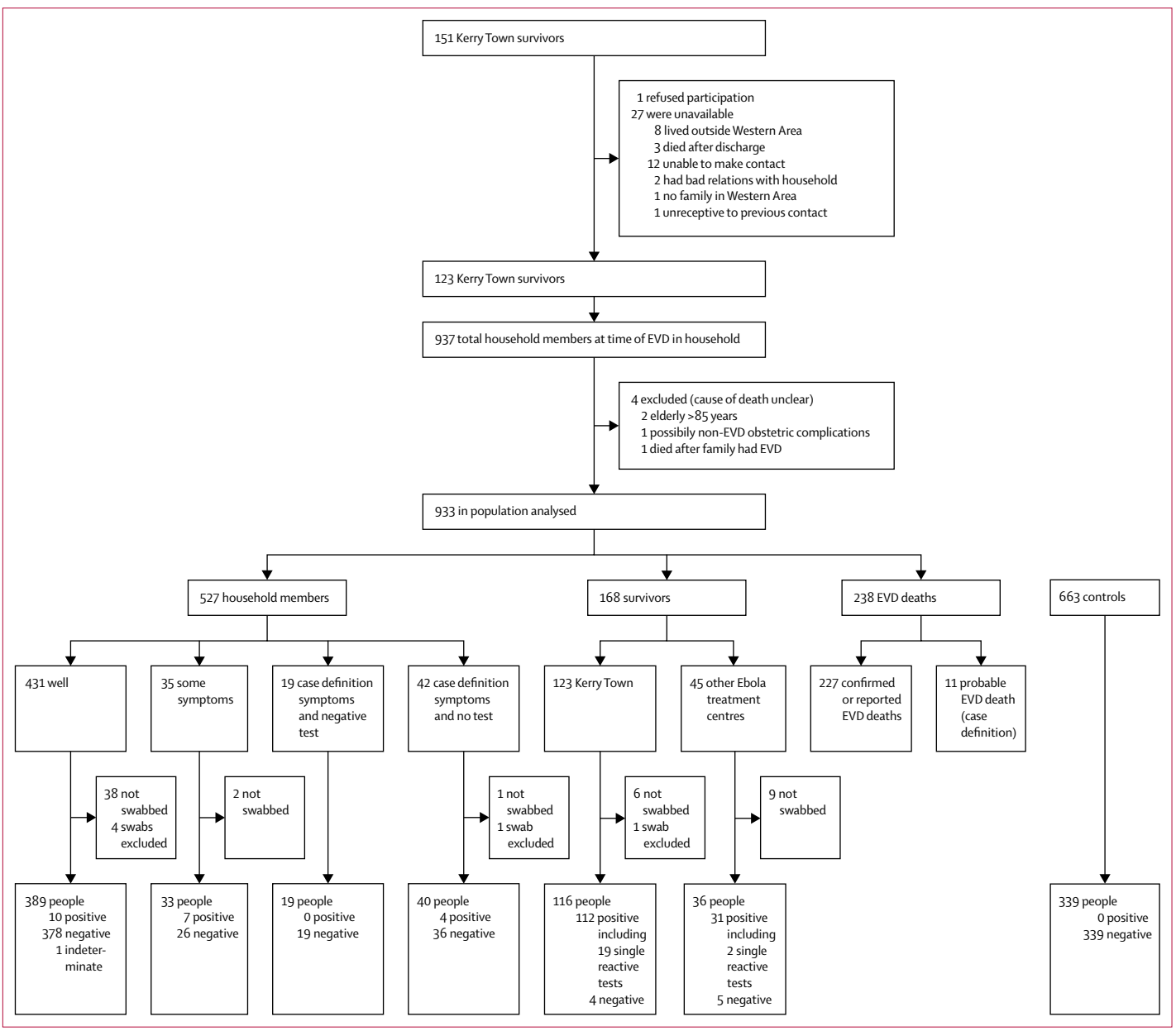

Figure 1: **Flow chart of study participants**
Households were defined as those who ate from the same pot. They included everyone who stayed there at the time Ebola was in the household, including those who were not normally resident. Of those not swabbed, most were absent; eight refused (all had been asymptomatic) and four had died since Ebola. Of the six excluded swabs, three were miscoded and three were not found. EVD=Ebola virus disease.

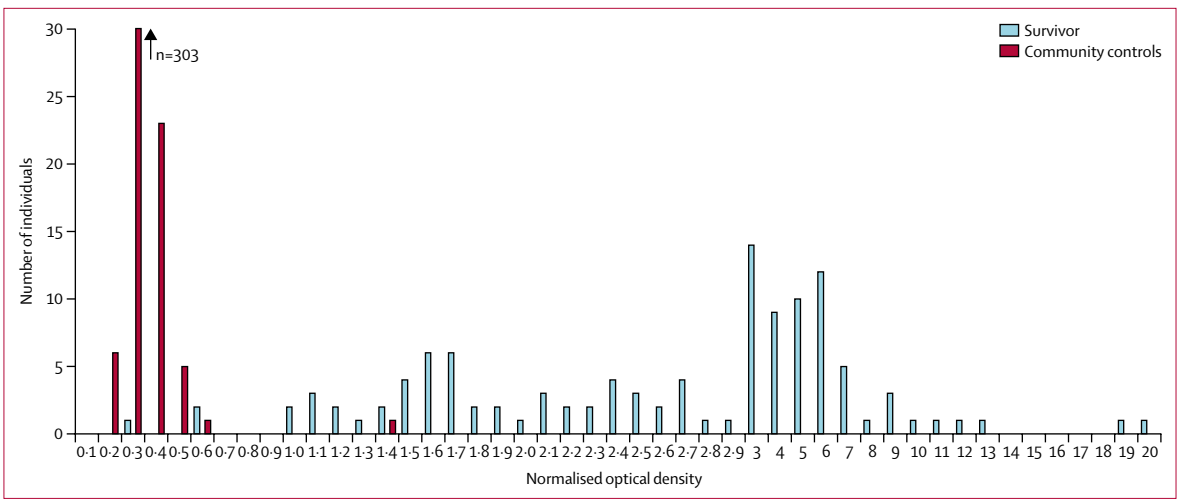

Figure 2: **Normalised optical densities of the first test in samples from 116 Kerry Town survivors and 339 Sierra Leone controls**

Statistical analysis

We assessed the sensitivity and specificity of the assay under field conditions using samples from PCR-confirmed Kerry Town EVD survivors and from the community controls.

For further analyses, individuals were defined as having been infected if their sample was reactive on two or more tests, uninfected if their sample was unreactive on one or more tests, and indeterminate if their sample had an equal number of reactive and unreactive tests. Two reactive tests were required to define infection to maximise specificity and hence positive predictive value, which is important because the prevalence of asymptomatic infection could be low. The CIs for the proportion positive were calculated using exact methods because of small numbers.

We assessed risk factors for infection among asymptomatic and symptomatic household members using χ^2 or Fisher's exact test as appropriate. We assessed confounding by age using logistic regression; further multivariable analysis was limited by the small number of events. Linear regression was used to assess the association of level of reactivity in the samples from survivors with time since admission and with age.

Data sharing

For the **raw data** see http://doi.org/10.17037/DATA.41

The raw data for this study are available online, by request.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The households of 123 of 151 Kerry Town survivors were included in the study. One survivor had subsequently died[15] but the household included another survivor so was visited. Of the remaining survivors, eight lived outside Western Area, three had died,[15] 16 were unavailable or uncontactable, and one refused to take part (figure 1).

The participating Kerry Town survivors lived in 91 households with 814 other household members, of whom 242 had died (227 from EVD, 11 from probable EVD, and four from unknown causes [who were excluded from further analyses]) and 45 were survivors from other facilities (figure 1). Of the 527 other household members, 96 had some symptoms around the time others in their household had EVD and 431 were asymptomatic. We collected 639 oral swabs from 153 survivors and 486 living household members, of which 633 (99·6%) could be analysed; only eight people (1·2%) refused to give a swab (figure 1). The mean age of the household members was 16·7 years (SD 14·2, range <1–84); 57% were female. The age and sex distribution of participating survivors and household members was similar to non-participants.

Oral swabs were collected from 663 community controls. Three people with possible Ebola virus exposure (two Ebola intervention workers and one funeral attendee) were excluded. Due to availability of test kits, we analysed the first 113 samples from each setting giving a total of 339 (mean age 19·0 years [SD 15·6, range <1–76], 53% female).

The distribution of normalised ODs (NODs) in the Kerry Town survivors and the community controls in the first test is shown in figure 2. From the Kerry Town survivors, 113 (97·4%) of 116 samples were reactive on the first test. 97 samples were retested: the three unreactive samples remained unreactive; one reactive sample was unreactive on retesting and on a third test (NODs 1·59, 0·97, and 0·69), and was considered negative; another reactive sample was unreactive on retesting and reactive on the third test (NODs 2·50, 0·76, and 1·01), and considered positive. All remaining initially reactive samples were

	Total	Positive			Negative				Indeterminate	IgG positive/total*	IgG positive (95% CI)
		RR	R	RUR	RUU	UUU	UU	U	RRUU		
Community controls	339	0	0	0	1	0	25	313	0	0/339	0·0% (0–1·08)
Kerry Town survivor	116	92	19	1	1	2	1	0	0	93/97	95·9% (89·8–98·9)
Household member: survivor from other Ebola treatment centre	36	29	2	0	0	2	3	0	0	29/34	85·3% (68·9–95·0)
Household member: asymptomatic	389	10	0	0	17	8	76	277	1†	10/388	2·6% (1·2–4·7)
Household member: symptomatic	92	10	0	1	1	2	8	70	0	11/92	12·0% (6·1–20·4)
Symptoms fitting case definition/no PCR test	40	3	0	1	1	1	3	31	0	4/40	10·0% (2·8–23·7)
Symptoms fitting case definition/PCR negative	19	0	0	0	0	1	0	18	0	0/19	0·0% (0–17·6)
Symptomatic not fitting case definition	33	7	0	0	0	0	5	21	0	7/33	21·2% (9·0–38·9)

Because of limited availability of kits, not all samples could be retested. We retested all positives (except some from known survivors of Ebola virus disease but including all those nearer the cutoff), all negatives from EVD survivors, and a sample of other negatives, prioritising those nearer the cutoff. We did third tests on any samples with discrepant results after two tests. For those samples with only one previous result, which were retested on the last available plate, we retested in duplicate in case any discrepancies arose. R=reactive. U=unreactive. *Total individuals; those with only a single reactive test available or indeterminate results excluded. †Retested because of borderline results; mean of all normalised optical densities 1·0 (SD 0·4; appendix p 5).

Table 1: **Prevalence of Ebola IgG positivity in samples from Ebola virus disease survivors, household contacts, and community controls, Sierra Leone, 2015**

repeatedly reactive and considered positive (table 1). Defining positive as two reactive tests gives a sensitivity of 95·9% (95% CI 89·8–98·9; 93 of 97 samples; table 2).

Among the community controls, all but one sample were unreactive on the initial test (338 [99·7%] of 339 samples). This sample was unreactive on second and third tests (NODs 1·41, 0·33, and 0·32). There were no further reactive results among 25 samples that were retested. Since no control sample was considered positive, specificity was 100% (95% CI 98·9–100).

Among those with duplicate tests, NODs were in good agreement in the different participant groups (appendix pp 2–4). Overall, comparison of the NODs of the first and second test using linear regression, gave an r^2 of 0·88.

Among the survivors from other treatment centres (for whom we did not have documented evidence of positive Ebola virus PCRs) 31 (86·1%) of 36 samples were positive for Ebola IgG. 40 (8·3%) of 481 samples from household contacts without diagnosed EVD were reactive on the first test. After subsequent tests, 21 were considered positive, 18 negative, and one indeterminate (table 1, appendix p 5). Among 389 asymptomatic contacts, ten (2·6%) of 388 were seropositive, compared with 11 (12·0%) of 92 symptomatic contacts (p=0·004). The asymptomatic infections were from different households, whereas two people with symptomatic undiagnosed infections were from the same household.

Asymptomatic infection was only seen in those older than 12 years. By contrast, among symptomatic contacts, seropositivity was highest in children younger than 5 years (four [26·7%] of 15) and in adults 30 years or older (six [35·3%] of 17) but undetected in teenagers and young adults (aged 10–29 years; table 3).

Level of exposure to Ebola correlated with seropositivity among asymptomatic and symptomatic contacts (table 3). Of the 12 individuals with direct contact with an EVD corpse who were not diagnosed with EVD themselves, four (33·3%) were infected, two asymptomatically. Among the 229 without known EVD with the three highest exposure levels (contact with corpse, body fluids, or wet cases), 16 (7%) were infected, seven asymptomatically. There were few socioeconomic factors associated with positivity (table 3). Associations with occupation and being household head were explained by age. 23 contacts had spouses who were EVD survivors so could potentially have been infected by sexual transmission after recovery. Two of these contacts were seropositive; both were male and had been symptomatic.

Among symptomatic contacts, neither the number of symptoms nor any individual symptom in the case definition, were associated with seropositivity, except for a non-significant correlation with red eyes (p=0·07; data not shown). The 11 seropositive undiagnosed symptomatic individuals were: a 1-year-old child with multiple symptoms who was not tested or admitted because of a nurses' strike; a 2-year-old child and a 9-year-old child with multiple symptoms who were not taken to a facility; three people with two symptoms (headache plus fatigue, loss of appetite or muscle or joint pain); and five people with single symptoms (abdominal pain, red eyes, hiccups, fever, or headache).

Overall, in these households there were 168 survivors and 238 EVD deaths reported at interview (figure 1), so

	Sensitivity (proportion of Kerry Town survivors reactive on test)		Specificity (proportion of community controls unreactive on test)	
	n/N	% (95% CI)	n/N	% (95% CI)
Single test	113/116	97·4% (92·6–99·5)	338/339	99·7% (98·4–99·99)
Confirmed	93/97	95·9% (89·8–98·9)	339/339	100% (98·9–100)

Results are presented on the basis of a single test, and using the rule that all reactive results should be confirmed by a second test.

Table 2: **Sensitivity and specificity of the oral fluid Ebola virus antibody test**

See Online for appendix

	Asymptomatic				Any symptoms			
	Total (n)	IgG positive			Total (n)	IgG positive		
		n	% (95% CI)	p		n	% (95% CI)	p
Total	389	10	2·6% (1·2–4·8)		92	11	12·0% (6·1–20·4)	
Sex								
Male	161	3	1·9% (0·0–5·3)	0·53	46	6	13·0% (4·9–26·3)	1·0
Female	228	7	3·1% (1·2–6·2)		46	5	10·9% (3·6–23·6)	
Age (years)								
<2	27	0	0·0% (0·0–12·8)	0·11	3	1	33·3% (0·8–90·6)	0·001
2–4	43	0	0·0% (0·0–8·2)	0·06 (trend)	12	3	25·0% (5·5–57·2)	
5–9	76	0	0·0% (0·0–4·7)		18	1	5·6% (0·1–27·3)	
10–14	73	4	5·5% (1·5–13·4)		14	0	0·0% (0·0–23·2)	
15–19	52	1	1·9% (0·0–10·3)		7	0	0·0% (0·0–41·0)	
20–29	67	2	3·0% (0·4–10·4)		21	0	0·0% (0·0–16·1)	
30–39	25	2	8·0% (1·0–26·0)		9	2	22·2% (2·8–60·1)	
40–49	11	1	9·1% (0·2–41·3)		5	2	40·0% (5·3–85·3)	
≥50	14	0	0·0% (0·0–23·2)		3	2	66·7% (9·4–99·2)	
Maximum exposure								
Handled corpse	10	2	20·0% (2·5–55·6)	0·003	2	2	100·0% (15·8–100)	0·06
Handled fluids	39	4	10·3% (2·9–24·2)	0·06 (trend)	17	1	5·9% (0·1–28·7)	
Direct wet contact	120	1	0·83% (0·0–4·6)		41	6	14·6% (5·6–29·2)	
Direct dry contact	68	0	0·0% (0·0–5·3)		13	1	7·7% (0·2–36·0)	
Indirect wet contact	11	0	0·0% (0·0–2·9)		2	0	0·0% (0·0–84·2)	
Indirect dry contact	52	1	1·9% (0·0–10·3)		11	0	0·0% (0·0–28·5)	
Minimal or no contact	89	2	2·2% (0·3–7·9)		6	1	16·7% (0·4–64·1)	
Occupation								
Unemployed or child	282	4	1·4% (0·4–3·6)	0·10	62	6	9·7% (3·6–19·9)	0·004
Health-care worker	9	0	0·0% (0·0–33·6)		1	0	0·0% (0·0–97·5)	
Manual work	85	4	4·7% (1·3–11·6)		22	1	4·5% (0·1–22·8)	
Non-manual work	10	1	10·0% (0·3–44·5)		6	4	66·7% (22·3–95·7)	
Status in household								
Head	23	1	4·3% (0·1–22·0)	0·46	14	5	35·7% (12·8–64·9)	0·01
Member	366	9	2·5% (1·1–4·6)		78	6	7·7% (2·9–16·0)	
Number of people in household								
1–5	24	0	0·0% (0·0–14·3)	0·63	7	1	14·3% (0·4–57·9)	0·90
6–10	126	5	4·0% (1·3–9·0)		39	5	12·8% (4·3–27·4)	
11–15	163	3	1·8% (3·8–5·3)		24	2	8·3% (1·0–27·0)	
>16	76	2	2·6% (3·2–9·2)		22	3	13·6% (2·9–34·9)	
Water available in household								
Sometimes	78	0	0·0% (0·0–4·6)	0·31	7	0	0·0% (0·0–41·0)	0·89
Most days	125	3	2·4% (0·5–6·9)		27	3	11·1% (2·4–29·2)	
Every day	183	6	3·3% (1·2–7·0)		58	8	13·8% (6·2–25·4)	
Soap available in household								
Sometimes	117	2	1·7% (0·2–6·0)	0·91	18	2	11·1% (1·4–34·7)	1·0
Most days	72	2	2·8% (0·3–9·7)		23	3	13·0% (2·8–33·6)	
Every day	197	5	2·5% (0·8–5·8)		51	6	11·8% (4·4–23·9)	
Latrine for household								
Shared or none	228	5	2·2% (0·7–5·0)	1·0	71	9	12·7% (6·0–22·7)	0·52
Household's own	158	4	2·5% (0·7–6·4)		21	2	9·5% (1·2–30·4)	
Crowding (people per room)								
High	89	3	3·4% (0·7–9·5)	0·68	25	4	16·0% (4·5–36·1)	0·16
Medium	253	5	2·0% (0·6–4·6)		60	5	8·3% (2·8–18·4)	
Low	44	1	2·3% (0·1–12·0)		7	2	28·6% (3·7–71·0)	

(Table 3 continues on next page)

	Asymptomatic				Any symptoms			
	Total (n)	IgG positive			Total (n)	IgG positive		
		n	% (95% CI)	p		n	% (95% CI)	p
(Continued from previous page)								
Spouse Ebola survivor								
No	372	10	2·7% (1·3–4·9)	1·0	86	9	10·5% (4·9–18·9)	0·15
Yes	17	0	0·0% (0·0–19·5)		6	2	33·3% (4·3–77·7)	
Household quarantined								
No	62	1	1·6% (0·0–8·7)	0·18	9	1	11·1% (0·3–48·2)	0·45
Yes	302	7	2·3% (0·9–4·7)		71	10	14·1% (7·0–24·4)	
Unknown	25	2	8·0% (1·0–26·0)		12	0	0·0% (0·0–26·5)	

p values from Fisher's exact test for heterogeneity are presented for all variables. p values from a non-parametric test for trend across ordered groups (an extension of the Wilcoxon rank-sum test) are presented where the proportions suggest a trend. In this table, the one sample from an asymptomatic individual with an indeterminate result was taken as negative. Age was missing for one person, occupation for four, household characteristics (water, soap, latrine and crowding) for three, and quarantine for 37.

Table 3: **Prevalence of Ebola IgG positivity in asymptomatic and symptomatic household members of Ebola virus disease survivors, Sierra Leone, 2015, by individual and household characteristics**

assuming seropositivity is a marker of Ebola virus infection, the ten asymptomatic and 11 symptomatic seropositive participants contributed 2·3% (ten of 427) and 2·6% (11 of 427) to Ebola virus infections, respectively. The contribution by age and exposure level is shown in figure 3 and appendix (p 9). In all age groups the proportion of infections that were asymptomatic was low, but it was higher in those aged 5–14 years (four [6·3%] of 64) than in those younger than 5 years (none [0%] of 53) and people aged 15 years or older (six [2·0%] of 307; p=0·07). The proportion of undiagnosed symptomatic infections was higher in those younger than 5 years (four [7·5%] of 53) than in those aged 5–14 years (one [1·6%] of 64) and those aged 15 years or older (six [2·0%] of 307; p=0·07).

Among those with positive tests, the NOD was similar in survivors and in those with asymptomatic (p=0·9) or missed symptomatic infections (p=0·7) in Wilcoxon rank-sum tests (appendix p 6).

Among survivors, no relation was seen between the magnitude of the NOD and the length of time since admission (appendix p 7) but the NOD was higher at younger ages (r^2=0·08, p<0·001; appendix p 8).

Discussion

The oral fluid IgG capture ELISA performed well in this field setting. The oral swabs were accepted by the population (only 1% refused) and were suitable for children and adults. The swabs required no processing before storage at –20°C, making them easy to use in field conditions. We optimised specificity by using a high cutoff (figure 2) and requiring two reactive results to confirm a positive; sensitivity remained high (95·9%).

Using this assay, 2·6% (ten of 388) of asymptomatic members of Ebola-affected households had evidence of Ebola virus infection. This result is lower than some household contact studies, but few such studies restricted examination to asymptomatic contacts, different assays were used, and the definition of contact varied. Excluding any symptomatic individuals, previous estimates were 2·5% (ten of 404) in Yambuku, Democratic Republic of the Congo, using an immunofluorescence assay;[16] 4·0% (four of 101) in Kikwit;[17] 21·4% (12 of 56) in Gabon;[18] 45·9% (11 of 24) among highly exposed contacts in Gabon;[4] 1·0% (two of 210) in Uganda,[3] and 6·5% (12 of 185) in Kono, Sierra Leone,[19] using different ELISAs. A preliminary report from Liberia studied 760 household members or sexual contacts; 13% were positive but it is not clear if all were asymptomatic or to which contact group they belonged.[20]

The higher proportion of asymptomatic infection in adolescents, and the higher reactivity levels in younger survivors are consistent with a lower risk of severe disease. Immunological differences between symptomatic and asymptomatically infected individuals, and between adults and children, have been noted previously.[17,21,22] The slight excess of missed symptomatic infections in children younger than 5 years is consistent with underdiagnosis in this group.[11] There was no evidence that any of the seropositive results were due to late transmission via semen:[23] only two spouses of EVD survivors were seropositive and both were male.

WHO guidelines for EVD survivor care[24] suggest that a positive IgG test could help define survivors if certificates (issued on discharge from a treatment centre) are missing, so a highly specific test is essential. An acceptable, sensitive, and specific assay would also assist vaccine studies, where knowledge of pre-existing immunity is important, and in identifying previously undiagnosed EVD cases who might have played a crucial part in transmission.

Testing the sensitivity assumed the Kerry Town survivors were correctly diagnosed. All four seronegative Kerry Town survivors were documented PCR-positive before admission; after admission, two (including the one with reactivity near the cutoff) had high-level PCR results, one had two low-level PCR results, and for the

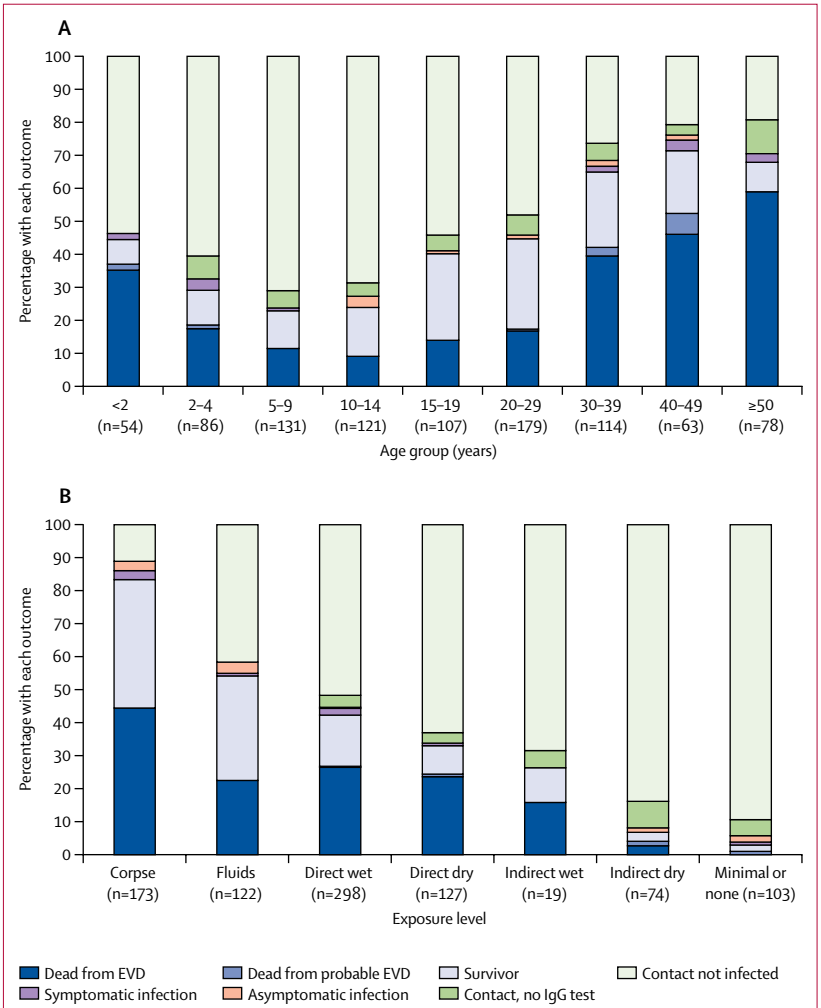

Figure 3: **Ebola manifestation and risk in households of survivors of Ebola virus disease (A) by age group in all members and (B) by exposure level (excluding the primary cases in each household)**
The primary cases were excluded for (B) so that the outcomes for each type of contact in Ebola-affected households could be seen. Information on deceased household members was provided at interview by the surviving household members. Exposure levels were determined from the interviews with all household members. Exposure levels are defined as follows: corpse, touched body of someone who died of EVD; fluids, direct contact with body fluids of a wet case (ie, an EVD case with diarrhoea, vomiting, or bleeding); direct wet, direct contact with a wet case (including nursing and personal care, sharing a bed, breastfeeding an EVD-positive child); direct dry, direct contact with a dry case (ie, an EVD case without wet symptoms); indirect wet, indirect contact with a wet case (eg, washing clothes or bed linen); indirect dry, indirect contact with a dry case; minimal or none, minimal contact (eg, shared meals) or no known contact. See Bower and colleagues[12] for details. EVD=Ebola virus disease.

one with the lowest reactivity (appendix p 5), who was aged in her 80s, we have no post-admission record of positive PCR results. Oral fluid containing insufficient IgG will fail to signal; this can only be checked by determining IgG concentrations, which was not available in this setting. We did not have paired serum samples from these individuals, though good correlation with oral fluid results has been shown previously.

The oral fluid samples were collected up to 10 months after exposure. A reduction in IgG concentrations is possible, though antibody persistence for several years has been noted previously,[17,25,26] and we found no evidence of a reduction (appendix p 7). It is theoretically possible that low level infections might have led to low concentrations of IgG that were not detected, which would underestimate the proportion of asymptomatic infections. However, in our study, above the cutoff, the NOD was similar in those with asymptomatic infection and in survivors (appendix p 6). We did not have enough test kits to re-test all those with initially unreactive results, but all 119 tested in duplicate remained unreactive.

Because our initial contact was through the community re-integration team, we only investigated survivor households. Survivors might be less infectious than those who die,[12,27–29] but 70% of households in the study had at least one EVD death and exposure levels were high: 47·6% (229 of 481) of household contacts without diagnosed EVD reported contact with corpses, body fluids, or wet cases, yet only 7·0% (16 of 229) of these were infected.

Accurate recall of symptoms is difficult. Forgetting or reluctance to admit previously unreported symptoms might overestimate the incidence of asymptomatic infection. Conversely, being in an EVD-affected household might have led to over-reporting of symptoms. During interviews family members would contribute details of the exposure and health of others, probably increasing recall accuracy.

In conclusion, we have used a non-invasive assay to show that asymptomatic Ebola virus infection occurs, but accounted for only a small proportion of infections, so would have little effect on herd immunity. It is unknown whether those with asymptomatic Ebola virus infection are infectious, or could harbour virus in the long term, like some survivors. In that respect, the low proportion of asymptomatic infections is reassuring because these transmissions would be challenging to prevent. We also identified missed symptomatic cases, some of which were mild. Many questions remain, including why some people escape infection or disease despite high exposure, and whether those asymptomatically infected will have any immunity in future outbreaks.

Contributors

JRG, HB, and FC designed the study with contributions from all other authors. RST and DS developed the assay, with SD, MGS, and JTS. HB and SJ led the fieldwork with MSB, AJK, OK, SHM, DS, and CT. CFH and CM led the laboratory work, with REGW and VC. HB and JRG did the analysis. JRG and HB led the writing with contributions from all other authors. All authors approved the final manuscript.

Declaration of interests

Save the Children International operated the Kerry Town Ebola Treatment Centre during the period under study, and employed SJ, MSB, AJK, OK, SHM, DS, and CT. FC was employed by Save the Children UK and was involved in commissioning the study and interpreting the findings. The authors declare no other competing interests.

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